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TITLE: Role of CYP1B1 in PAH-DNA Adduct Formation and Breast Cancer Risk

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13. ABSTRACT (Maximum 200 Words) Interindividual variation in carcinogen metabolism is an important determinant of susceptibility to various cancers. In breast tissue, the major enzyme involved in metabolizing polycyclic aromatic hydrocarbons (PAHs) to reactive intermediates appears to be the cytochrome P450 enzyme CYP1B1. High CYP1B1 enzyme levels may result in increased formation of PAH-DNA adducts in breast tissue and lead to subsequent development of breast cancer. Gene expression analysis captures an important convergence of multiple genetic and environmental factors on metabolic enzyme levels. This study tests hypotheses pertaining to underlying molecular mechanisms of the relationship between PAH exposure and breast cancer risk: 1. Increased CYP1B1 gene expression is associated with increased risk of invasive breast cancer; 2. Increased PAH-DNA adduct formation is associated with increased risk of invasive breast cancer; 3. Increased CYP1B1 gene expression is associated with increased PAH-DNA adduct formation in breast cells; and 4. The positive association between CYP1B1 gene expression and increased risk of invasive breast cancer is not due to genotype variation. Given that certain environmental exposures, such as PAHs, appear to be important breast cancer risk factors, establishing biologic pathways through which these agents act will provide new insights for disease prevention.				
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INTRODUCTION

This study investigates underlying molecular mechanisms of the relationship between PAH exposure and breast cancer risk. In breast tissue, the major enzyme involved in metabolizing PAHs to reactive intermediates appears to be the cytochrome P450 enzyme CYP1B1. High CYP1B1 enzyme levels may result in increased formation of PAH-DNA adducts in breast tissue and lead to subsequent development of breast cancer. Gene expression analysis captures an important convergence of multiple genetic and environmental factors on metabolic enzyme levels. Using a clinic-based case-control design, breast tissue will be obtained from female patients undergoing either mastectomy or reduction mammoplasty surgery in Porto Alegre, Brazil. PAH exposure and potential confounding factor data will be collected for all cases and controls via medical chart review and interviewer-administered questionnaire. To characterize molecular level interindividual variation in PAH metabolism, *CYP1B1* gene expression and PAH-DNA adducts will be measured in the surgically obtained breast epithelial cells from at least 37 mastectomy cases and 74 reduction mammoplasty controls.

BODY

New FWA requirements have delayed obtaining final IRB approval and have delayed the start of the study. Obtaining an FWA for the collaborating Brazilian institution has been challenging. The home institution of our Brazilian collaborator does not have a registered Institutional Review Board (IRB). The Brazilian National Ethics Committee does not have a registered IRB. The University of California at Berkeley (UCB) IRB agreed to serve as the registered IRB for the Brazilian institution's FWA application provided that a person with knowledge of local context of the research participated in the review of the protocol. Department of Health and Services/Office for Human Research Protection (OHRP) has accepted this arrangement. The UCB IRB has completed its review of the study protocol with Dr. Jose Roberto Goldim, an ethicist from Porto Alegre, Brazil. The Brazilian institution's FWA have been submitted to OHRP, and we await final approval.

Meanwhile, we have completed a revised draft of the questionnaire that includes a reformatted residence and occupational history sections and extensive PAH exposure questions. Items pertaining to known breast cancer risk factors have been revised to correspond to those items being used in an on-going population-based case-control study in California. After receiving full IRB approval from all of the necessary institutions, testing of the revised questionnaire will commence.

We have recently developed improved methods for measuring CYP1B1 expression levels using real-time RT-PCR and recommend that these methods be used in place of those described in the original proposal. Rather than normalizing CYP1B1 gene expression to beta-actin, we propose to normalize the CYP1B1 gene expression of each specimen to the cytokeratin-18 gene, a specific epithelial cell marker. The cytokeratin-18 gene will serve as a control for both the epithelial cell content and the quantity of RNA isolated from each specimen. A PCR-based assay for calibrator-normalized relative quantitation of CYP1B1 levels was developed using a rapid micro-capillary cyler with real-time product detection by fluorescence (LightCycler,

Roche Molecular Biochemicals). The ratio of CYP1B1 to cyotkeratin-18 levels of each specimen will be compared to a calibrator sample. The method uses RNA isolated from primary cultures of a human mammary epithelial cell line, HMEC 184, as a calibrator. The calibrator will be included in each amplification to correct for differing amplification efficiencies between the two gene targets. The CYP1B1 expression level (corrected for epithelial cell content) will then be expressed as an N-fold difference between the specimen and the calibrator.

The cDNA from each case and each control is amplified with both cyotkeratin-18 primers (1) and with CYP1B1 primers (2) in two separate reactions. Both primer sets are designed to span an intron, thus excluding amplification of any contaminating genomic DNA. These primers generate products of 158 bp for CYP1B1, and 114 bp for cyotkeratin-18. (All primer sequences were confirmed for gene specificity by conducting a GenBank database sequence search.) Amplification conditions (such as annealing temperatures, Mg²⁺ concentrations, and primer concentrations) are optimized to produce only specific products as detected by a melting curve analysis performed at the end of each amplification cycle. The melting curve is unique to each product and is dependent on length and nucleotide sequence of the DNA product; it serves to identify each product formed during PCR. The CYP1B1 levels in unknown samples are then determined from the first cycle of the PCR run in which the signal can be distinguished from the background.

KEY RESEARCH ACCOMPLISHMENTS

- Achieved consensus on the wording of the informed consent forms and protocol between DOD HSRRB and UCB IRB.
- Assisted with coordination of a special subcommittee review of the study protocol with UCB IRB and a Brazilian ethicist.
- Submitted FWA application to OHRP for our Brazilian collaborator's institution. (We have not yet heard back from OHRP regarding the status of this application other than that they did receive the application).
- Revised previous pilot questionnaire to include items to assess PAH exposure.
- Developed improved laboratory methods for measuring *CYP1B1* expression using an epithelial cell marker.

REPORTABLE OUTCOMES

Goth-Goldstein R, Caleffi M, Russell ML, Li D, Erdmann CA. Role of CYP1B1 in PAH-DNA adduct formation and breast cancer risk. Abstract. Era of Hope Department of Defense Breast Cancer Research Program Meeting. Orlando, Florida. September 25-28, 2002.

CONCLUSIONS

Because of the potential important role of CYP1B1 in the activation of PAHs to carcinogenic intermediates, it is hypothesized that high *CYP1B1* expression could result in high levels of PAH-

DNA adducts. We look forward to receiving final IRB approval for this project and to begin collecting data soon.

REFERENCES

None.

APPENDICES

None.